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CHRISTENSON, STEVEN D.
STANDAGE, SCOTT

- <120> GENE CLUSTER FOR PRODUCTION OF THE ENEDIYNE ANTITUMOR ANTIBIOTIC C-1027
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- <150> 60/115,434
- <151> 1999-01-06
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C-1027 gene cluster sequence

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105

100

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Leu Ala Pro Asn Val Pro Tyr Ala Ala Ser Lys Ala Gly Ser Asp Leu 145 150 155 160

Met Ala Leu Ala Trp His Arg Thr Arg Gly Leu Asp Val Val Thr 165 170 175

Arg Cys Thr Asn Asn Tyr Gly Pro Tyr Gln Tyr Pro Glu Lys Val Ile 180 185 190

Pro Leu Phe Val Thr Asn Ile Leu Asp Gly Leu Arg Val Pro Leu Tyr 195 200 205

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Leu Lys Glu Ala Ala Gly Leu Leu Asp Ala Val Gly 325 330

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<211> 521

<212> PRT

<213> Streptomyces globisporus

<220>

 $\mathbf{r} = \mathbf{r}_{-1} = \mathbf{r}$

<223> sgcB

<400> 114

Met Thr Ala Val Lys Glu Pro Thr Ser Arg Ala Gly Arg Arg Glu Trp
1 5 10 15

Ile Ala Leu Val Val Leu Ser Leu Pro Thr Met Leu Leu Met Leu Asp

• • •

Ile	Asn	Val 35	Leu	Met	Leu	Ala	Leu 40	Pro	Gln	Leu	Ser	Glu 45	Asp	Leu	Gly
Ala	Ser 50	Ser	Thr	Gln	Gln	Leu 55	Trp	Ile	Thr	Asp	Ile 60	Tyr	Gly	Phe	Ala
Ile 65	Ala	Gly	Phe	Leu	Val 70	Thr	Met	Gly	Thr	Leu 75	Gly	Asp	Arg	Ile	Gly 80
Arg	Arg	Arg	Leu	Leu 85	Leu	Gly	Gly	Ala	Ala 90	Val	Phe	Ala	Val	Val 95	Ser
Val	Val	Ala	Ala 100	Phe	Ser	Asp	Ser	Ala 105	Ala	Met	Leu	Val	Val 110	Ser	Arg
Ala	Val	Leu 115	Gly	Val	Ala	Gly	Ala 120	Thr	Val	Met	Pro	Ser 125	Thr	Leu	Ala
Leu	Ile 130	Ser	Asn	Met	Phe	Glu 135	Asp	Pro	Lys	Glu	Arg 140	Gly	Thr	Ala	Ile
Ala 145	Met	Trp	Ala	Ser	Ala 150	Met	Met	Ala	Gly	Val 155	Ala	Leu	Gly	Pro	Ala 160
Val	Gly	Gly	Leu	Val 165	Leu	Ala	Ala	Phe	Trp 170	Trp	Gly	Ser	Val	Phe 175	Leu
Ile	Ala	Val	Pro 180	Val	Met	Leu	Leu	Val 185	Val	Val	Thr	Gly	Pro 190	Val	Leu
Leu	Thr	Glu 195	Ser	Arg	Asp	Pro	Asp 200	Ala	Gly	Arg	Leu	Asp 205	Leu	Leu	Ser
Ala	Gly 210	Leu	Ser	Leu	Ala	Thr 215	Val	Leu	Pro	Val	Ile 220	Tyr	Gly	Leu	Lys
Glu 225	Leu	Ala	Arg	Thr	Gly 230	Trp	Asp	Pro	Leu	Ala 235	Ala	Gly	Ala	Val	Val 240
Leu	Gly	Val	Ile	Phe 245	Gly	Ala	Leu	Phe	Val 250	Gln	Arg	Gln	Arg	Arg 255	Leu
Ala	Asp	Pro	Met 260	Leu	Asp	Leu	Gly	Leu 265	Phe	Ala	Asp	Arg	Thr 270	Leu	Arg
Ala	Gly	Leu 275	Thr	Val	Ser	Leu	Val 280	Asn	Ala	Val	Ile	Met 285	Gly	Gly	Thr
Gly	Leu 290	Met	Val	Ala	Leu	Tyr 295	Leu	Gln	Thr	Ile	Ala 300	Gly	His	Ser	Pro
Leu	Ala	Ala	Gly	Leu	Trp	Leu	Leu	Ile	Pro	Ala	Cys	Met	Leu	Val	Val

Gly Val Gln Leu Ser Asn Leu Leu Ala Gln Arg Met Pro Pro Ser Arg 325 330 335

Val Leu Gly Gly Leu Leu Ile Ala Ala Val Gly Gln Leu Leu Ile 340 345 350

Thr Gln Val Asp Thr Glu Asp Thr Ala Leu Leu Ile Ala Ala Thr Thr 355 360 365

Leu Ile Tyr Phe Gly Ala Ser Pro Val Gly Pro Ile Thr Thr Gly Ala 370 375 380

Ile Met Gly Ala Ala Pro Pro Glu Lys Ala Gly Ala Ala Ser Ser Leu 385 390 395 400

Ser Ala Thr Gly Gly Glu Phe Gly Val Ala Leu Gly Ile Ala Gly Leu
405 410 415

Gly Ser Leu Gly Thr Val Val Tyr Ser Ala Gly Val Glu Val Pro Asp 420 425 430

Ala Ala Gly Pro Ala Asp Ala Asp Ala Gln Glu Ser Ile Ala Gly
435 440 445

Ala Leu His Thr Ala Gly Gln Leu Ala Pro Gly Ser Ala Asp Ala Leu 450 455 460

Leu Asp Ser Ala Arg Ala Ala Phe Thr Ser Gly Val Gln Ser Val Ala

465 470 475 480 Ala Val Cys Ala Val Phe Ser Leu Ala Leu Ala Val Leu Ile Gly Thr 485 490 495

Arg Leu Arg Asp Ile Ser Ala Met Asp His Gly His Gly Glu Glu Pro 500 505 510

Ala Glu Asn Asp Ala Gln Pro Ala Thr 515 520

<210> 115

. . .

<211> 329

<212> PRT

<213> Saccharopolyspora erythraea

<400> 115

Met Arg Val Leu Val Thr Gly Gly Ala Gly Phe Ile Gly Ser His Tyr
1 10 15

Val Arg Gln Leu Leu Gly Gly Ala Tyr Pro Ala Phe Ala Gly Ala Asp 20 25 30

Val Val Leu Asp Lys Leu Thr Tyr Ala Gly Asn Glu Glu Asn Leu 35 40 45

Arg Pro Val Ala Asp Asp Pro Arg Phe Arg Phe Val Arg Gly Asp Ile

• 61 •

Cys Glu Trp Asp Val Val Ser Glu Val Met Arg Glu Val Asp Val Val 65 70 75 80

Val His Phe Ala Ala Glu Thr His Val Asp Arg Ser Ile Leu Gly Ala 85 90 95

Ser Asp Phe Val Val Thr Asn Val Val Gly Thr Asn Thr Leu Leu Gln 100 105 110

Gly Ala Leu Ala Ala Asn Val Ser Lys Phe Val His Val Ser Thr Asp

Glu Val Tyr Gly Thr Ile Glu His Gly Ser Trp Pro Glu Asp His Leu 130 135 140

Leu Glu Pro Asn Ser Pro Tyr Ser Ala Ala Lys Ala Gly Ser Asp Leu 145 150 155 160

Ile Ala Arg Ala Tyr His Arg Thr His Gly Leu Pro Val Cys Ile Thr 165 170 175

Arg Cys Ser Asn Asn Tyr Gly Pro Tyr Gln Phe Pro Glu Lys Val Leu 180 185 190

Pro Leu Phe Ile Thr Asn Leu Met Asp Gly Arg Arg Val Pro Leu Tyr 195 200 205

Gly Asp Gly Leu Asn Val Arg Asp Trp Leu His Val Thr Asp His Cys 210 215 220

Arg Gly Ile Gln Leu Val Ala Glu Ser Gly Arg Ala Gly Glu Ile Tyr 225 230 235 240

Asn Ile Gly Gly Gly Thr Glu Leu Thr Asn Lys Glu Leu Thr Glu Arg 245 250 255

Val Leu Glu Leu Met Gly Gln Asp Trp Ser Met Val Gln Pro Val Thr 260 265 270

Asp Arg Lys Gly His Asp Arg Arg Tyr Ser Val Asp His Thr Lys Ile 275 280 285

Ser Glu Glu Leu Gly Tyr Glu Pro Val Val Pro Phe Glu Arg Gly Leu 290 295 300

Ala Glu Thr Ile Glu Trp Tyr Arg Asp Asn Arg Ala Trp Trp Glu Pro 305 310 315 320

Leu Lys Ser Ala Pro Asp Gly Gly Lys 325 <211> 333

9 e j gr

<212> PRT

<213> Streptomyces fradiae

<400> 116

Met Arg Val Leu Val Thr Gly Gly Ala Gly Phe Ile Gly Ser His Phe 1 5 10 15

Thr Gly Gln Leu Leu Thr Gly Ala Tyr Pro Asp Leu Gly Ala Thr Arg
20 25 30

Thr Val Val Leu Asp Lys Leu Thr Tyr Ala Gly Asn Pro Ala Asn Leu 35 40 45

Glu His Val Ala Gly His Pro Asp Leu Glu Phe Val Arg Gly Asp Ile 50 55 60

Ala Asp His Gly Trp Trp Arg Arg Leu Met Glu Gly Val Gly Leu Val 65 70 75 80

Val His Phe Ala Ala Glu Ser His Val Asp Arg Ser Ile Glu Ser Ser 85 90 95

Glu Ala Phe Val Arg Thr Asn Val Glu Gly Thr Arg Val Leu Leu Gln
100 105 110

Ala Ala Val Asp Ala Gly Val Gly Arg Phe Val His Ile Ser Thr Asp 115 120 125

Glu Val Tyr Gly Ser Ile Ala Glu Gly Ser Trp Pro Glu Asp His Pro 130 135 140

Val Ala Pro Asn Ser Pro Tyr Ala Ala Thr Lys Lys Ala Ser Asp Leu 145 150 155 160

Leu Ala Leu Ala Tyr His Arg Thr Tyr Gly Leu Asp Val Arg Val Thr 165 \$170 175

Arg Cys Ser Asn Asn Tyr Gly Pro Arg Gln Tyr Pro Glu Lys Ala Val 180 185 190

Pro Leu Phe Thr Thr Asn Leu Leu Asp Gly Leu Pro Val Pro Leu Tyr 195 200 205

Gly Asp Gly Gly Asn Thr Arg Glu Trp Leu His Val Asp Asp His Cys 210 215 220

Arg Gly Val Ala Leu Val Gly Ala Gly Gly Arg Pro Gly Val Ile Tyr 225 230 235 240

Asn Ile Gly Gly Thr Glu Leu Thr Asn Ala Glu Leu Thr Asp Arg 245 250 255

Ile Leu Glu Leu Cys Gly Ala Asp Arg Ser Ala Leu Arg Arg Val Ala 260 265 270

Asp Arg Pro Gly His Asp Arg Arg Tyr Ser Val Asp Thr Thr Lys Ile 275 280 285

Arg Glu Glu Leu Gly Tyr Ala Pro Arg Thr Gly Ile Thr Glu Gly Leu 290 295 300

Ala Gly Thr Val Ala Trp Tyr Arg Asp Asn Arg Ala Trp Trp Glu Pro 305 310 315 320

Leu Lys Arg Ser Pro Gly Gly Arg Glu Leu Glu Arg Ala 325 330

<210> 117

9 1 j P

<211> 331

<212> PRT

<213> Streptomyces argillaceus

<400> 117

Met Thr Thr Thr Ser Ile Leu Val Thr Gly Gly Ala Gly Phe Ile Gly 1 5 10 15

Ser His Tyr Val Arg Thr Leu Leu Gly Pro Arg Gly Val Pro Asp Val 20 25 30

Thr Val Thr Val Leu Asp Lys Leu Thr Tyr Ala Gly Thr Leu Thr Asn 35 40 45

Leu Ala Glu Val Ser Asp Ser Asp Arg Phe Arg Phe Val Arg Gly Asp 50 55 60

Ile Cys Asp Ala Pro Leu Val Asp Asp Leu Leu Ala Val His Asp Gln 65 70 75 80

Val Val His Phe Ala Ala Glu Ser His Val Asp Arg Ser Ile Leu Gly 85 90 95

Ala Ala Asp Phe Val Arg Thr Asn Val Thr Gly Thr Gln Thr Leu Leu 100 105 110

Asp Ala Ala Leu Arg Gln Gly Ile Glu Thr Phe Val His Ile Ser Thr 115 120 125

Asp Glu Val Tyr Gly Ser Ile Asp Ala Gly Ser Trp Pro Glu Thr Ala 130 135 140

Pro Val Ser Pro Asn Ser Leu Tyr Ser Ala Ala Lys Ala Ser Ser Asp 145 150 155 160

Leu Val Ala Leu Ala Tyr His Arg Thr His Gly Leu Asp Val Arg Val
165 170 175

Thr Arg Cys Ser Asn Asn Tyr Gly Ser His Gln Phe Pro Glu Lys Val 180 185 190

Ile Pro Leu Phe Val Thr Ser Leu Leu Asp Gly Arg Glu Val Pro Leu

195 200 205

Tyr Gly Asp Gly Thr Asn Val Arg Asp Trp Leu His Val Asp Asp His 210 215

Val Arg Ala Ile Glu Leu Val Arg Thr Gly Gly Arg Ala Gly Glu Val 230 235

Tyr Asn Ile Gly Gly Gly Thr Glu Leu Ser Asn Lys Glu Leu Thr Gln 250

Leu Leu Asp Ala Cys Gly Ala Gly Trp Asp Arg Val Arg Tyr Val 260

Thr Asp Arg Lys Gly His Asp Arg Arg Tyr Ser Val Asp Cys Thr Lys 280 285

Ile Arg Arg Glu Leu Gly Tyr Arg Pro Ala Arg Glu Phe Gly Asp Ala 290 295

Leu Ala Glu Thr Val Ala Trp Tyr Arg His His Arg Ala Trp Trp Glu 305

Pro Leu Thr Arg Ala Tyr Gly Ala Val Ala Ala 325

<210> 118

<211> 6

4

<212> PRT

<213> Artificial Sequence

<223> Description of Artificial Sequence: 6-His tag

<400> 118

His His His His His